Alameda County RESOURCE CONSERVATION DISTRICT

Progress Report (2020):

Expanding Early-season California Milkweed (*Asclepias californica*) for Monarch Conservation in Alameda County Rangelands

Funding from the Alameda Fish and Game Commission, granted in 2019, enabled the Alameda County Resource Conservation District (ACRCD) to partner with East Bay Regional Parks (EBRPD) and the Natural Resources Conservation Service (NRCS) to identify locations where California milkweed (*Asclepias californica*) occurs and to collect seed that was grown by the Watershed Nursery for outplanting on public lands and on private ranches in Alameda County. 2019 progress was summarized in the previously submitted ACRCD report, "Progress Report: Expanding Early-season California Milkweed (*Asclepias californica*) for Monarch Conservation in Alameda County Rangelands". 2019 accomplishments are briefly re-presented here for context; ACRCD's summary of 2020 activities beings on page 2.

Summary of activities completed in 2019:

- California milkweed distribution
    - ACRCD and project partners developed the California Milkweed in the East Bay project in iNaturalist, allowing citizen scientists to collaboratively document occurrences of *A. californica* in Contra Costa County and in Alameda County; this project continues to provide data on *A. californica* observations, improving our understanding of the species habitat requirements and current distribution, in connection with existing records on Calflora.
    - ACRCD and project partners reported the first record of *A. californica* (one stand) on San Francisco Public Utility property in the Alameda Creek Watershed.
- California milkweed protection
    - NRCS consulted with the East Bay Chapter of the California Native Plant Society to bring *A. californica* onto their radar as a rare local species, aiming to increase sighting records and prioritize protection.
- California milkweed seed collection and propagation
    - Based on above-mentioned observations, ACRCD and project partners visited five locations from which to collect *A. californica* seed for species propagation: (1) EBRPD Morgan Territory Regional Preserve (no plants observed); (2) Round Valley Regional Preserve (two plants observed, but no seed available in August 2019); (3) Las Trampas Regional Wilderness (no *A. californica* observed, but *A. speciosa* recorded); (4) private ranch on Mines Rd., East Alameda Co. (17 plants observed, seed collected); and (5) Black Diamond Mines Regional Preserve (8 plants observed, seed collected from 6).

Because late rains caused a delay in bloom and seed production in 2019, ACRCD and project partners delayed seed germination to 2020. This delay was necessary to maximize the growth window for *A. californica* seedlings, allowing outplantings sufficient root structure to increase their chances of survival.

Summary of activities completed in 2020

- *A. californica* seed from Mines Rd. and Black Diamond Mines collections was sown and propagated by The Watershed Nursery; data were collected to assess seed germination and transplant survival rate of seedlings treated under different conditions.
- A drafted, associated propagation and seed survival study (Sardinas et al.; p. 3), is being prepared to summarize this work and will be published as a white paper or in an appropriate local journal, in order to contribute to propagation guidance for this species. Black Diamond Mines germination rates were particularly low, thought to be related to later collection of seed pods from this location.
- NRCS and TWN staff delivered 136 propagated *A. californica* to three ranches along Mines Road, including greenhouse storage, from which final delivery to two additional ranches is scheduled after additional winter rains. For ideal planting conditions, additional winter rains are desired to avoid drought-induced mortality of plants placed in the ground.

Planting of these seedlings is anticipated in early 2021 and will complete grant deliverable as initially described in the ACRCD grant proposal "Expanding Early-season California Milkweed (*Asclepias californica*) for Monarch Conservation in Alameda County Rangelands", dated 15 Feb 2019.

Figure 1. *Delivery of California milkweed to rancher (M. McElfresh) Watershed Nursery (D. Benner) and NRCS (not pictured), 15 Dec 2020, Mines Rd., Alameda Co., CA. Photo credit – J. Charbonneau, NRCS.by The*

Figure 2. California milkweed delivered to ranch on Mines Rd., Dec. 2020, propagated from 2019 seedling collection.

Seed Germination Rates of California Milkweed (*Asclepias californica*) Following Hot Water Scarification

Introduction

Monarchs butterflies (*Danaus plexippus plexippus*) that are part of the western population, which occur west of the Rocky Mountains, are leaving their costal overwintering sites earlier in the season due to climate change and microclimate changes within groves related to tree senesce (Crone et al. 2019; Espeset et al 2016; Pelton et al. 2019). They are therefore also seeking breeding locations earlier in the year. As a multi-generational species, the success of the first breeding generation is linked to the viability of the population in later generations. Researchers have identified the lack of oviposition sites on milkweed (*Asclepias* sp.), the monarch's host plant, as key to the conservation of their species (Peltonet al. 2019).

Only a few milkweed species – *A. californica*, *A. cordifolia*, *A. erosa,* and *A. vestita*— leaf out during the winter period that coincides with the first round of breeding, between January and March (Borders and Lee-Mäder 2011). Based on records from CalFlora and iNaturalist, these species occur in lower abundance, with more narrow distributions, than more common, widespread milkweed species in California, such as narrow leaf (*A. fasicularis*) and showy (*A. speciosa*) milkweed. In addition, these early season species are seldom available commercially or utilized in restoration projects. There is also less known about techniques that enhance the propagation success of these species. Expanding production and availability of these species may be able to help address the recent catastrophic decline of the western monarch population.

Methods

We focused on California milkweed (*A. californica;* ASCA3) because it is the only early-season milkweed in the East Bay, where partners for this project are located. ASCA3 has been posited to be the "most important milkweed species for monarchs in establishing their spring generation in the west" (Brower etal. 1984). ASCA3 has large maroon to magenta flowers that bloom between late March and late June, depending on the local climate and seasonal rainfall. It is the earliest blooming of the three early-season milkweed species and can emerge as early as December, though January – march is more typical (Harris 1986). During its bloom period, the plant has an upright posture, usually growing between 12 to 24 inches tall. When it goes to seed, it becomes more prostrate. The leaves of ACSA3 are tomentose. Its seed pods typically split open and disperse seeds by August. Like all milkweeds, its sap is a milky latex that contains cardenolides, the chemical compound that monarch caterpillars bioaccumulate, making them unpalatable to predators.

We collected ASCA3 at two locations in the East Bay, on a private ranch in the Livermore area and at Black Diamond Mines, part of the East Bay Regional Parks District (EBRPD). Both locations were in seed zone 35-40 Deg F/ 3-6 (TRM Seed Zone Map). Seeds from the ranch were collected in mid-July 2019 andfrom EBRPD in early August 2019. The stands were variable sizes, with the private ranch containing over17 plants and the regional park only 7. The stand on the ranch was located in a grassland with scatteredoaks on a southwest facing slope while the other was in a grassland on an eastern facing slope. Both sites were grazed by cattle, and we did note some browsing of the seed pods and leaves. The ranch population was visited in June and approximately 1/3 of developing seed pods were bagged with nylon stocking material to protect them from herbivory and capture seeds in case of pod burst. The property owner continued to bag newly developing seed pods and to collect seed between mid-July through August. At Black Diamond Mines, there were collected from burst seed pods. Milkweed bugs

(*Oncopeltus fasciatus*), which are voracious seed predators, were also present. A total of 8.31 grams of seed were collected from the Black Diamond Mines population and 113.5 grams from the private ranch population. Seeds were cleaned then kept in cold storage until trials began in January 2020.

We trialed various treatments and timings to determine germination success rate in a native plant nursery located in Richmond, CA (seed zone 40-45 Deg F/ 3-6). Treatments included: no treatment, cold stratification (maintaining seeds in cold, moist conditions to mimic winter conditions), or a hot water treatment followed by cold stratification (hot-cold). We replicated these treatments in the winter and spring, initiated in January and April respectively. The winter sowing cold stratification treatment consisted of placing seeds in slightly moistened Sunshine Mix #5 in a Ziploc bag which was placed in a refrigerator at 14°C. The hot treatment consisted of pouring water heated to 77°C over the seeds and leaving seeds to soak for 24 hrs, straining, then placing in a Ziploc bag with slightly moistened Sunshine Mix #5 in a refrigerator. Seeds were checked weekly for germination.

Our plan was to keep seeds in stratification until germination was observed, expected to occur between 2-6 weeks. Molding of the seeds in stratification was observed in the 'winter' phase of production. Seeds were removed from stratification, rinsed, soaked in a 1% bleach solution for 1 minute, and put back into stratification with new media. Because of this molding, the spring stratification was adjusted from waiting until germination was observed (~49 days) to sowing all seeds after 2 weeks of stratification.

After germination, we evaluated the impact of transplanting on survival. We either sowed seeds into shallow sowing flats that necessitated transplanting of seedlings or direct sowed the seeds into target containers, D16 tubes (Stuewe & Sons, 2" wide, 7" deep). In two cases, we sowed seeds in deepflats to allow the seedlings to be transplanted later. We included these in the direct sow category because there was not sufficient replication to separate out the results. Media for all treatments was heat-treated and mixed with 20% perlite.

Because there were so few seeds collected from the Black Diamond Mines site, all of them were used in the winter phase. We therefore excluded this site from our analyses. We do, however, report their germination rates.

Plants were outplanted in the field in December 2020, thus we cannot share rates of field survival at this time but plan to track their success as well.

We analyzed the data using a binomial model with package lmer in R Studio (version 4.0.3). We evaluated the effects of treatment and season on germinations rates of plants. Separately, we looked atthe impact of transplanting on plant survival (through October 2020). In both models we included a random effect of seed lot.

Results

We collected 96 seeds from the stand at EBRPD and 1,233 from the private ranch. Only 11% of the seeds from the EBRPD site germinated—from the cold treatment. No seeds from the other lots germinated. Allof those did germinate, however, survived to outplanting. Seeds from the private ranch had a mean germination rate of 33%, though there was high variation (± 16% SD). An additional 9% of plants were lost before outplanting.

The average proportion of plants that germinated was higher for those that received the hot-cold treatment (44%, $p < 0.001$; figure 1; table 1) than cold stratification only (29%) or no treatment (16%).

The number days from treatment to germination averaged 38 (± 16 SD) for the winter phase and 20 (± 9 SD) for the spring phase. The number of seeds that germinated was slightly higher in the spring (39 ± 15 SD; $p < 0.05$) than the winter (18 ± 10 SD).

Transplanting did not impact the survival rate after germinated plants were potted into containers, despite the average number being higher for directly sown plants (29 ± 14 SD) than plants transplantedinto 4packs (15 ± 16 SD). This is likely due to the high variation in the number of plants that survived.

Discussion

The variation in number of seeds we collected at the different sites could be related to stand size or timing and method of seed collection. Larger stands may experience enhanced cross-pollination, leading to increased seed viability. We were unable to bag the seed pods at the regional park, which meant thata significant portion of the seeds had already dropped from the ripe pods. In addition, the remaining seeds were not protected from milkweed bugs, whose piercing-sucking mouthparts are used to suck thecontents from the seed making them inviable (Borders and Lee-Mäder 2014). Testing seed viability by floating seeds and only treating sinkers, has also been proposed as a method to eliminate non-viable seeds (pers. comm. D. Kopp, USFS). Identifying stands early and bagging pods prior to dehiscal is likely an important factor in ensuring seed viability.

For ASCA3, cold stratification alone was not significantly different from no treatment. Previous studies on other milkweed species found that cold stratification was sufficient to induce increased germination rates (described in Borders and Lee-Mäder 2014). The addition of the hot water treatment lead to a significant increase in seed germination in the seeds we collected. Milkweed seeds have a hard outer coat that protect the seed during its dormant period. Previous studies have evaluated whether mechanical (e.g., using sandpaper or nicking with a razor) or chemical scarification can induce increasedgerminations rates, to varied effects (for additional description, see Borders and Lee-Mäder 2014). The hot water treatment we used is easier to use when working with large number of seeds, as labor is typically the limiting factor when treating high numbers of seeds.

Despite the success of the hot-cold treatment, we nevertheless saw an attrition rate of > 50% of all seed collected. Milkweed has the reputation of being finicky and having high die-off rates. It is therefore necessary to collect *at least* twice the amount of seeds for the plants desired for a given restoration project.

Sowing in the spring was slightly more successful than a mid-winter sowing, though the reason for this is unclear. Although ASCA3 is an early-blooming species, it is perennial and dies back every year before resprouting in the late winter. It is unknown what time of year the seeds germinate in the wild, but it could be later in spring than when an established plant resprouts. Seeds may also take longer to germinate in different regions based on degree days or other factors.

We did not detect a difference in the survival of plants that were transplanted versus direct sown. There was high variation in transplant success. While transplant shock can be common in some species, it is unknown whether this is the case for ASCA3. There are anecdotal reports of the species bring challenging to maintain in containers through its dormant period. Conversations with staff from a handful of nurseries in California led to recommendations to outplant the same year as propagated, when possible. More research into conditions that support seedling survival after germination is warranted.

Conclusion

The hot water treatment plus cold stratification is promising for enhancing germination of ASCA3 seeds. Additional trials of seed collected in different locations should be conducted, however, before widespread adoption.

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Note: This paper has not been through peer review.

Figures

Figure 1. Germination rates were higher for the hot water plus cold stratification (hot-cold; B) treatment than for cold only or no treatment, which were indistinguishable from one another (A).

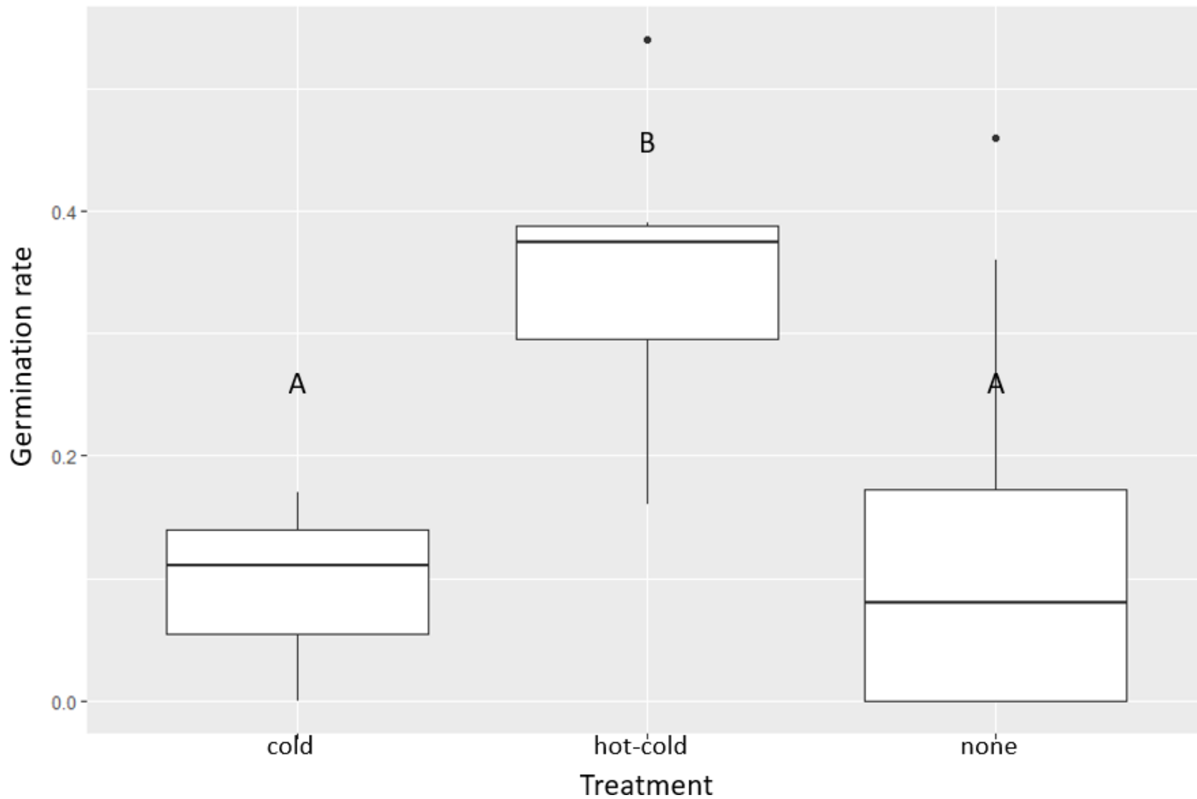


Figure 2. Germination rates were slightly higher when plants were sown in spring.

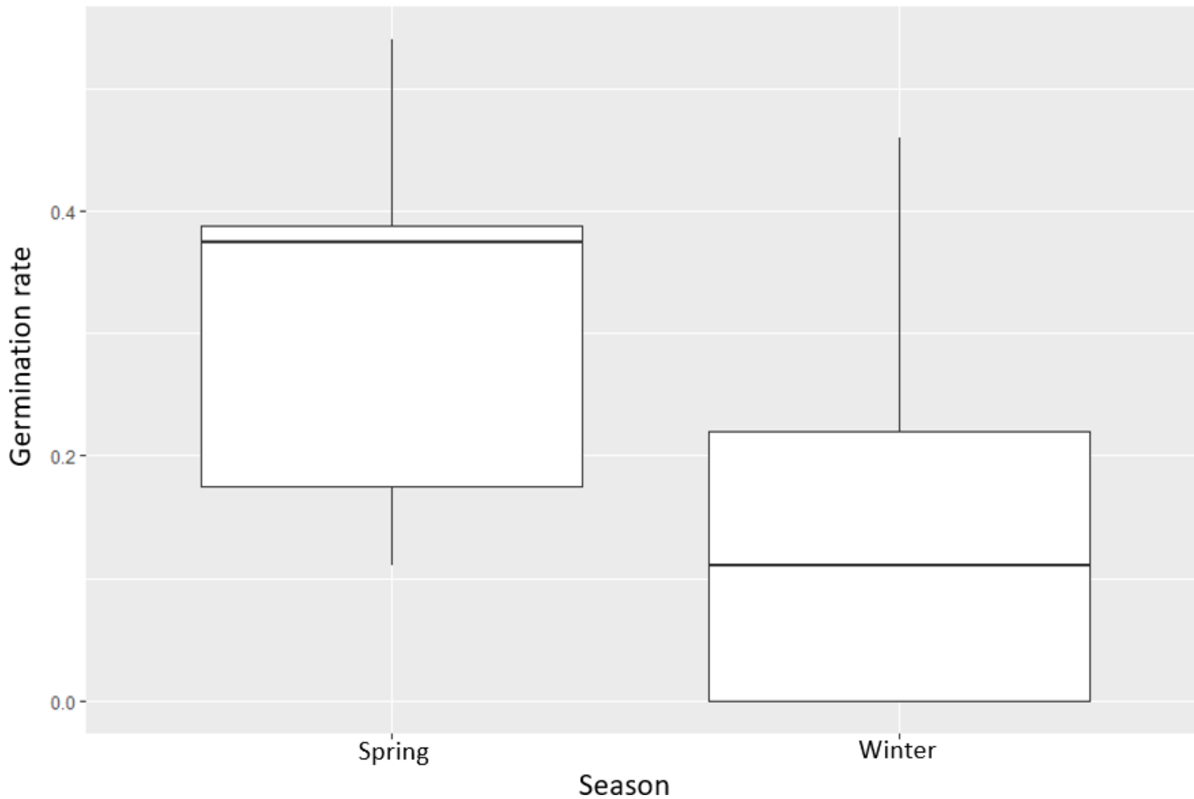
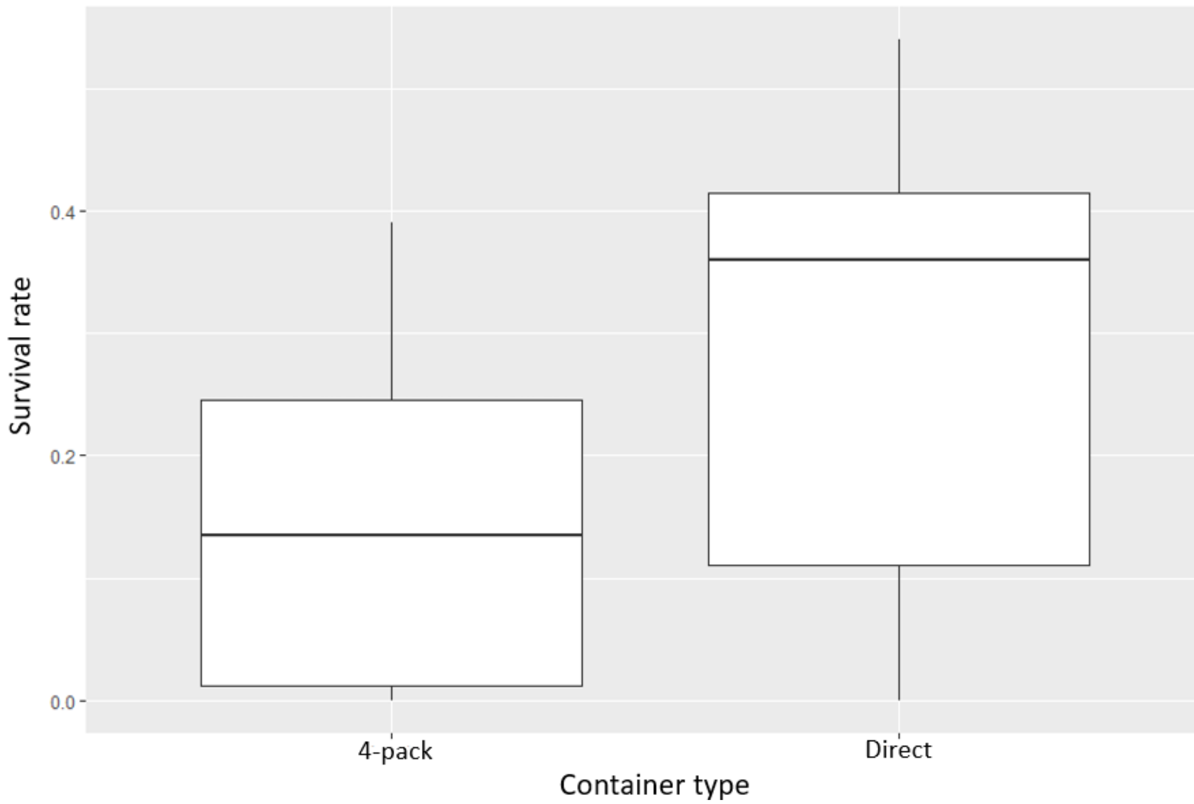


Figure 3. Container type, which influenced the need to transplant (4-pack) or not (direct sown), did not impact survival rates.



Tables

Table 1. Summary of results from binomial models testing the effects of season and treatment ongermination rates and container type on transplant survival.

Response variable	**Explanatory variable**	**Estimate**	**Z-value**	**P-value**
Germination rate	Winter	0.30	2.17	**<0.05**
	Cold	0.29	1.23	0.216
	Hold-cold	0.96	0.36	**<0.001**
Transplant survival	Container type	0.08	0.60	0.56

Photos

Photo 1. Bagged seed heads of California milkweed. Note that not all the seed pods were bagged on each plant to allow for natural dispersal. Credit- Diana Benner

Photo 2. Close up of a green seed ASCA3 pod. The milky drops are the latex. Credit- Diana Benner.


Photo 3. ASCA3 seedlings that were directly sown into D16 containers.


Photo 4. Root system for ASCA3 in a D16 pot at the end of the first growing season. Credit- Diana Benner.